

CLAIMS:

1. A method of making a no wash bead based assay, the method comprising:

preparing a first reagent comprising a buffer;

preparing a second reagent comprising a protein;

preparing beads of preselected size and having a coefficient of variation less than 5%, including washing the beads in the buffer to form a bead-buffer matrix and reducing the surfactancy of the beads to an effective amount;

adding an antigen for detecting the presence of a target species to the bead-buffer matrix such that the antigen attaches to the beads to form a bead-antigen mixture, the surfactancy of the beads facilitating attachment of the antigen thereto;

adding buffer to the bead-antigen mixture and thereafter incubating the mixture; and

adding second reagent to the bead-antigen mixture to reduce or eliminate non-specific binding sites.

2. A method as claimed in claim 1 wherein the first reagent is a carbonate buffer.

3. A method as claimed in claim 2 wherein the carbonate buffer

has a pH in the range of 9.0 - 10.0.

4. A method as claimed in claim 3 wherein the carbonate buffer has a pH of 9.6.

5

5. A method as claimed in claim 1 wherein the second reagent is bovine serum albumin (BSA).

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600
605
610
615
620
625
630
635
640
645
650
655
660
665
670
675
680
685
690
695
700
705
710
715
720
725
730
735
740
745
750
755
760
765
770
775
780
785
790
795
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995
1000

6. A method as claimed in claim 5 wherein the BSA comprises a 0.1 - 5.0% BSA in saline.

7. A method as claimed in claim 6 wherein the BSA is a 0.5% BSA in saline.

8. A method as claimed in claim 1 wherein the size of the beads is selected from one or more of the group consisting of 3 μ latex beads, 4 μ latex beads, 5 μ latex beads, 6 μ latex beads, 7 μ latex beads, 8 μ latex beads, 9 μ latex beads and 10 μ latex beads.

9. A method as claimed in claim 8 wherein the beads are selected so as to have a coefficient of variation not exceeding 5%.

10. A method as claimed in claim 9 wherein the beads are selected so as to have a coefficient of variation not exceeding 1.3%.

11. A method as claimed in claim 8 wherein multiple sizes of beads are selected.

12. A method as claimed in claim 1 wherein the antigens added are selected from the group consisting of RNP/SM, SM, SS-A, SS-B, SCL-70 and dsDNA.

13. A method as claimed in claim 1 wherein the antigens are selected from one of more of the group consisting of histones, lipids, viral antibodies, viral antigens, bacterial antibodies, bacterial antigens, recombinant proteins, and cellular antigens.

14. A method as claimed in claim 1 wherein the surfactancy of the beads is reduced to no more than 5% in order to enhance the ability to coat the beads with antigens.

15. A method as claimed in claim 14 wherein the surfactancy is no more than 0.5% of the beads.

16. A method as claimed in claim 1 wherein the bead-based assay is prepared in a flat-bottom container.

17. A method as claimed in claim 1 wherein the bead-buffer matrix is subjected to at least one prewashing step.

18. A method as claimed in claim 1 further comprising the step of centrifuging the bead-buffer matrix and the bead-antigen mixture, and the resuspension thereof.

19. A method as claimed in claim 1 further comprising the step of vortexing the bead-buffer matrix and the bead-antigen mixture, and the resuspension thereof.

20. A method of manufacturing a no wash kit for carrying out a bead based assay for testing for the presence of a target substance, the method comprising:

preparing a first reagent comprising a buffer;

preparing a second reagent comprising a protein;

preparing a third reagent comprising an indicator antibody, the third reagent being selected for its ability to identify the target substance;

preparing beads of preselected size and having a coefficient of variation less than 5%, including washing the beads in the buffer to form a bead-buffer matrix and reducing the surfactancy of the beads to an effective amount;

5 adding an antigen for detecting the presence of the target substance to the bead-buffer matrix such that the antigen attaches to the beads to form a bead-antigen mixture, the surfactancy of the beads facilitating attachment of the antigen thereto;

10 adding buffer to the bead-antigen mixture and thereafter incubating the mixture;

105873866-060401 adding second reagent to the bead-antigen mixture to reduce or eliminate non-specific binding sites;

 placing the bead-antigen mixture in a first container for use in an assay test procedure; and

15 placing the third reagent in a second container for use during the test procedure, the third reagent being used after the target substance has attached to the bead for the purpose of identifying the target substance.

20 21. A method as claimed in claim 20 wherein the third reagent comprises goat anti-human Ig.

22. A method as claimed in claim 20 comprising a plurality of bead-antigen mixtures, wherein a plurality of different antigens are each attached to a bead of different size.

23. A method as claimed in claim 22 wherein the bead sizes are selected from the following: 3 μ latex beads, 4 μ latex beads, 5 μ latex beads, 6 μ latex beads, 7 μ latex beads, 8 μ latex beads, 9 μ latex beads and 10 μ latex beads.

24. A method as claimed in claim 22 wherein one or more of the antigens are selected from the group consisting of RNP/SM, SM, SS-A, SS-B, SCL-70 and dsDNA.

25. A no wash kit for carrying out a bead based assay for testing for the presence of a target substance, the kit comprising a bead based assay prepared according to the method of claim 1, and a detector assay reagent comprising an indicator antibody, the detector assay reagent being selected for its ability to identify the target substance.

26. A no wash bead based assay for testing for the presence of a target substance, the assay being prepared according to the method

of claim 1.

0997396-030401
FOUO "99E450